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Research Article

A study on genetic relationship between *Allium sativum* L. and *Scadoxus multiflorus* (Martyn) Raf. of Amaryllidaceae

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Abstract

The present investigation has been focussed on the inter-chromosomal and intra-chromosomal asymmetry data of *Allium sativum* and *Scadoxus multiflorus* of Amaryllidaceae using quali-quantitative and quantitative methods. The quail-quantitative analysis is strictly ratio based and it revealed that the karyotype of *A. sativum* and *S. multiflorus* belonged to category 1A and 3C respectively indicating only the degree of asymmetry. The coefficient of variation of chromosome length (CV_{CL}) and the mean centromeric asymmetry (M_{CA}) were the estimators of quantitative analysis and the data showed that the inter- and intra-chromosomal asymmetry were comparatively high in *S. multiflorus* than that of *A. sativum*. Further, the genetic distance between these two species is clearly visible in the bi-dimensional scattered plot from the derived M_{CA} and CV_{CL} data.

Keywords: Bi-dimensional scattered plot; inter-chromosomal asymmetry; intra-chromosomal asymmetry; karyotype

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Introduction

Karyological studies of *Allium sativum* L. and *Scadoxus multiflorus* (Martyn) Raf. (=*Haemanthus multiflorus*) belonging to the family Amaryllidaceae had been carried out by different researchers from time to time (1-6). *A. sativum*, commonly known as garlic, is extensively used as spice in the Indian subcontinent (1). On the other hand, *S. multiflorus* is extensively cultivated as ornamental plant for its beautiful flower (6). Though Konvicka and Levan (1) observed significant differences in the karyotype of different clones of *A. sativum*, previous cytological studies revealed that the karyotype of *A. sativum* is symmetrical in nature (3, 5). Moreover, comparative analysis of karyotype of *Allium* spp. had also yielded the order of chromosomal evolution in related species (7). On the contrary, asymmetrical karyotype was found to be the inherent characteristics of *S. multiflorus* (4, 6). However, in order to evaluate the karyotype symmetry or asymmetry of a species one has to deal with both inter-chromosomal and intra-chromosomal asymmetries of the somatic

chromosome complements of species studied and usually these are determined by quali-quantitative method (8) and/or quantitative methods based on statistical approach (9,10). Stebbins (8) quali-quantitative method of determining karyotype asymmetry is based on the ratio of largest and smallest chromosome in a karyotype (inter-chromosomal asymmetry) and proportion of chromosome arm ratio (L/S) <2:1 (intra-chromosomal asymmetry). Recently, Peruzzi and Eroglu (11) have developed another model in which coefficient of variation of chromosome length (CV_{CL}) is coupled with a new parameter called mean centromeric asymmetry (M_{CA}). Accordingly, the karyotype asymmetry relationship among different species/ families/tribes was represented by drawing scatter plot putting M_{CA} data in X axis and CV_{CL} data in Y axis. An attempt has therefore been made to establish the genetic relationship between *A. sativum* and *S. multiflorus* of Amaryllidaceae using CV_{CL} and M_{CA} data of each species.

Materials and Methods

Bulbs of *Allium sativum* and *Scadoxus multiflorus* were collected from Madhupur (23°43'28.22"N, 91°13'33.70"E), Sepahijala, Tripura and grown in the experimental garden, Department of Botany, Tripura University. Somatic chromosome study of *A. sativum* and *S. multiflorus* was carried out with modified aceto-orcein staining technique (12). Young root tips of each species were pre-treated separately in a saturated solution of para dichloro-benzene (p-DB) at 12-15°C for 5 hrs. The root tips were then washed with distilled water and kept in acidulated alcohol mixture of 1NHCL and Ethanol (1:1) for 30 mins. Thereafter, root tips were kept in 45% acetic acid for 15 mins, stained with 2% aceto-orcein:1NHCl (9:1) mixture for 2 hrs. and finally squashed in 45% acetic acid. The well spread metaphase plate was captured using Carl Zeiss make AXIO Lab A1 microscope located in the department of Botany, Tripura University and Zen software was used for determining the length of short and long arm of each individual chromosome of the species studied. In quali-quantitative method (8) inter chromosomal and intra chromosomal indices of each species were calculated by the following formula:

Inter chromosomal asymmetry index

$$= \frac{Length\ of\ the\ largest\ chromosome\ (\mu m)}{Length\ of\ the\ smallest\ chromosome\ (\mu m)}$$

Intra-chromosomal asymmetry index = Proportion of chromosomes with arm ratio (L/S) < 2:1

In quantitative method (11), inter chromosomal and intra chromosomal indices were calculated by deriving the coefficient of variation of chromosome length (CV_{CL}) and the Mean Centromeric Asymmetry (M_{CA}) of each species where,

$$CV_{CL} = A_2 \times 100 \text{ and}$$

$$A_2(9) = \frac{Standard\ deviation\ of\ the\ chromosome\ length}{Mean\ of\ chromosome\ length}$$

$$M_{CA} = A \times 100 \text{ and}$$

$$A(10) = \left[\frac{\sum \frac{Long\ arm\ (L) - Short\ arm\ (S)}{Long\ arm\ (L) + Short\ arm\ (S)}}{n} \right]$$

Finally, the karyotype asymmetry relationship between the species was represented by drawing scatter plot putting M_{CA} data in X-axis and CV_{CL} data in Y-axis (11).

Results and Discussion

The somatic chromosome number of *Allium sativum* was found to be 2n=16 (Fig. 1) which corroborates previous findings (1, 3). Numerical data of the karyotype of *A. sativum* revealed that its chromosomes are either metacentric or submetacentric in nature (Fig. 1) and their length ranged from 9.24 µm to 16.17 µm (Table 1). This also indicates the absence of acrocentric or telocentric chromosomes in its karyotype. Based on quali-quantitative estimation, the inter-chromosomal and intra-chromosomal indices of *A. sativum* were found to be 1.75 and 1.00

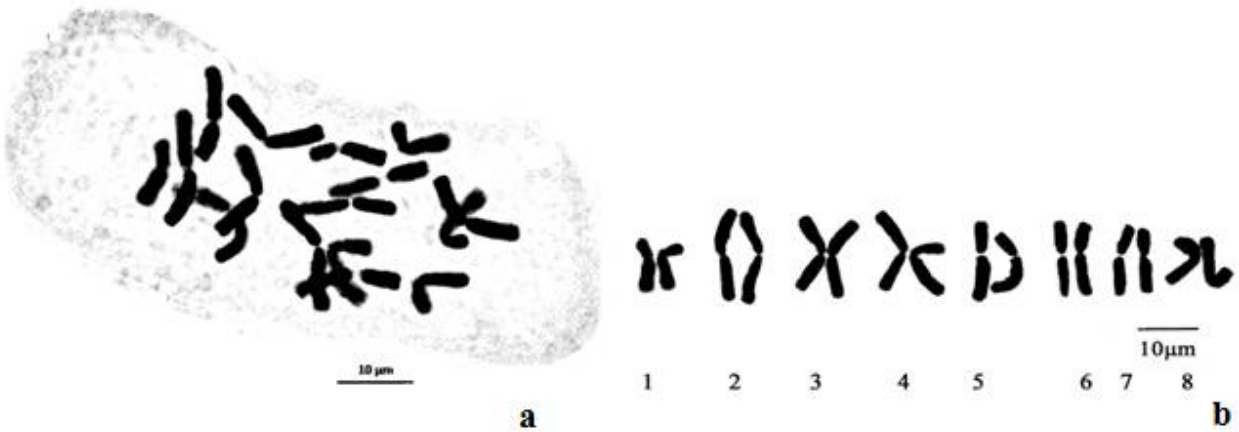

Fig. 1. (a) Mitotic metaphase plate showing 2n = 16 chromo-somes **(b)** karyogram of *Allium sativum*

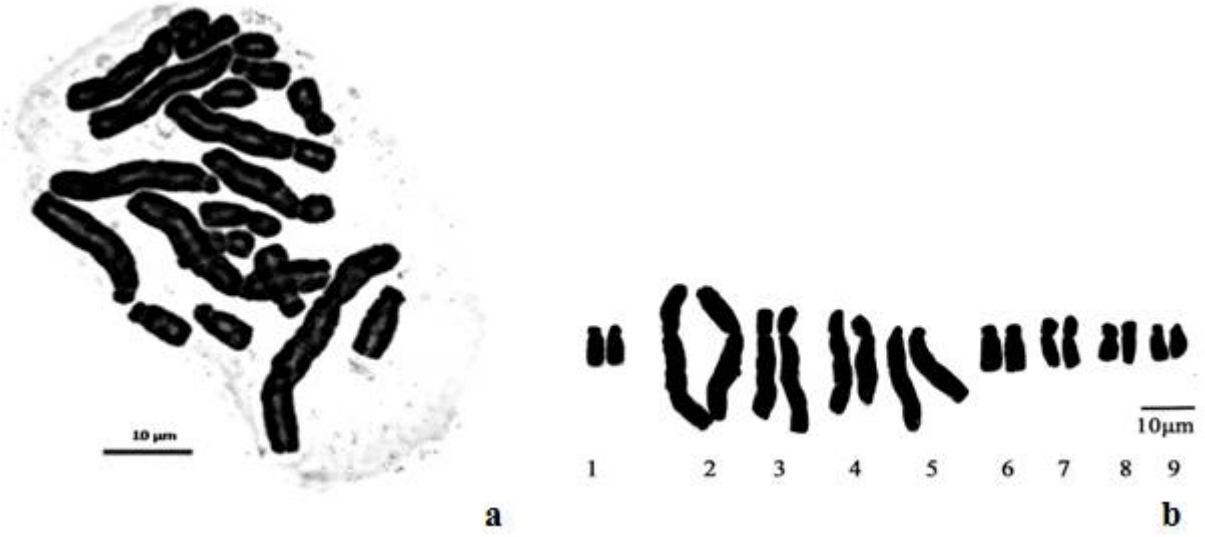

Fig.2. (a). Mitotic metaphase plate showing 2n = 18 chromosomes **(b)** karyogram of *Scadoxus multiflorus*

Table 1. Numerical data of karyotype of *Allium sativum* and *Scadoxus multiflorus*

Name of the species	Somatic Chromosome number	Total chromosome length (µm)	TF%	Length of largest and smallest chromosome (µm)	Largest to smallest chromosome ratio	Proportion of chromosomes with arm ratio <2:1	CV_{CL}	M_{CA}
Allium sativum	2n=16	203.74	41.92	16.17; 9.24	1.75	1.00	19.87	12.20
Scadoxus multiflorus	2n=18	304.40	25.67	38.00; 6.50	5.84	0.22	59.00	43.90

Table 2. Karyotype asymmetry index (8) of *Allium sativum* and *Scadoxus multiflorus*

Ratio of length of largest chromosome and smallest chromosome	Proportion of chromosomes with arm ratio (L/S) < 2:1			
	1.00	0.51 – 0.99	0.10 – 0.50	0.00
	(1)	(2)	(3)	(4)
< 2:1 – A	*Allium sativum* – 1A			
2:1 to 4:1 - B				
> 4:1 – C			*Scadoxus multiflorus* – 3C	

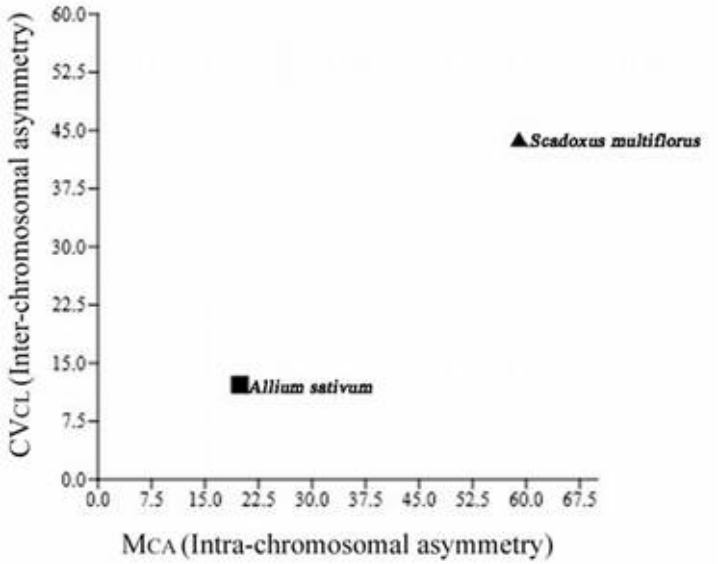

Fig. 3. Scatter plot derived from karyotype asymmetry data, M_{CA} (x axis) and CV_{CL} (y axis) of *Allium sativum* and *Scadoxus multiflorus*

respectively. Thus, the karyotype of *A. sativum* falls under Stebbins category 1A (Table 2). Our results are also in agreement with previous reports (1, 5). Simultaneously, we also estimated the defined inter-chromosomal and intra-chromosomal indices (CV_{CL} and M_{CA}) through quantitative methods, which were not reported previously. The value of CV_{CL} and M_{CA} in *A. sativum*

was estimated for the first time as 19.87 and 12.20 respectively (Table 1). In the present study, the somatic chromosome count of *Scadoxus multiflorus* was found to be 2n=18 chromosomes (Fig.2). From the numerical data it was observed that the karyotype of *S. multiflorus* had four pairs of large chromosomes and five pairs of small chromosomes (Fig. 2) and this shows the karyotype is composed of two distinct classes with respect to the size of the chromosomes indicating the bimodal nature. The length of chromosomes of *S. multiflorus* ranged from 6.50 µm to 38.00 µm (Table 1) and the TF% (13) value (Table 1) justified the presence of acrocentric and telocentric chromosomes (4, 6). Through quali-quantitative estimation, the inter- and intra-chromosomal asymmetry indices were found to be 5.84 and 0.22 (Table 1) respectively and so under Stebbins categorization the karyotype of *S. multiflorus* falls under 3C which was not reported earlier. In contrast, the quantitative estimation of inter and intra-chromosomal asymmetry indices revealed that the value of CV_{CL} and M_{CA} of *S. multiflorus* were 59.0 and 43.90, (Table 1) respectively. Thus, quali-quantitative asymmetry data of *A. sativum* and *S. multiflorus* indicate only the varying degree of asymmetry of their karyotype but the genetic distance between the two species cannot be assessed from such relationship. On the contrary, quantitative data of inter- and intra- chromosomal asymmetry index when plotted in bi-dimensional scattered plot the genetic distance between *A. sativum* and *S. multiflorus* is clearly reflected in the graph (Fig. 3) indicating that inter- and intra-chromosomal indices were comparatively high in *S. multiflorus* than those of *A. sativum*. This also suggests that CV_{CL} and M_{CA} are the determinants of inter-chromosomal and intra-chromosomal asymmetry. Similar such findings were also reported by previous researchers (14, 15) where the karyotype asymmetry relationship among organisms has been explained by means of bi-dimensional scattered plot of M_{CA} and CV_{CL} data (11).

Conclusion

The karyotype asymmetry data estimated in *A. sativum* and *S. multiflorus* clearly establishes the genetic distance between these two species and this suggests that the coefficient of variation of chromosome length (CV_{CL}) and the mean centromeric asymmetry (M_{CA}) data could be easily used in bi-dimensional scatter plot in order to establish the genetic relationship among different species/families in future cyto-taxonomical work.

Competing Interests

The authors have no conflict of interests.

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Authors' contributions

HDP and KS designed the experiment and wrote the manuscript.

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